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BIOASSAY OF NITHIAZIDE FOR POSSIBLE CARCINOGENICITY

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BIOASSAY OF

NITHIAZIDE

FOR POSSIBLE CARCINOGENICITY

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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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REPORT ON THE BIOASSAY OF NITHIAZIDE
FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of nithiazide conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of nithiazide was conducted by Litton Bionetics, Inc., Bethesda, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. S. M. Garner (4,5) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly. Chemical analysis was performed by Midwest Research Institute (6) and the analytical results were reviewed by Dr. N. Zimmerman (7).

Histopathologic examinations were performed by Dr. A. DePaoli (4), Dr. P. Hildebrandt (4), Dr. R. Montali (4), Dr. H. Seibold (4), and Dr. N. J. Wosu (4) at Litton Bionetics, Inc., the pathology narratives were written by Dr. A. DePaoli (4), and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (8).

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (9); the statistical analysis was performed by Mr. W. W. Belew (7,10) and Mr. R. M. Helfand (7), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (11).

This report was prepared at METREK, a Division of The MITRE Corporation (7) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (7), task leader Ms. P. Walker (7), senior biologist Mr. M. Morse (7), biochemist Mr. S. C. Drill (7), and technical editor Ms. P. A. Miller (7). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1,12), Dr. R. A. Griesemer (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,13), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

The bioassay of nithiazide for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. Nithiazide was administered in the diet, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low concentrations of nithiazide utilized were, respectively, 1250 and 625 ppm for rats and 5000 and 2500 ppm for mice. Dosed rats received feed containing nithiazide for 38 weeks, and as a result of a shortage of nithiazide, the animals were not fed the dosed feed for the next 9 weeks. The dosed feed diet was then resumed and continued for 56 weeks, after which time a 1-week observation period followed. Dosed mice received feed containing nithiazide for 61 weeks and, due to a shortage of nithiazide, the animals were not fed dosed feed for the next 9 weeks. The dosed feed diet was then resumed and continued for 33 weeks, followed by a 1-week observation period. Twenty animals of each sex and species were placed on test as controls.

In both species, adequate numbers of animals survived sufficiently long to be at risk from late-developing tumors. There was no significant positive association between dosage and mortality for either rats or mice. Compound-related mean body weight depression occurred in both sexes of each species.

Statistically significant incidences of hepatocellular adenomas and carcinomas were found in high dose male mice but not in female mice. Although the increased incidences of these tumors in dosed female mice were not statistically significant, the evidence presented was strongly suggestive of carcinogenicity to the liver in female B6C3F1 mice. Statistically significant increased incidences of a combination of mammary and skin fibroadenomas and cystadenomas NOS were found in the high dose female rats. No unusual tumors were observed in either species.

Under the conditions of this bioassay, nithiazide was carcinogenic in male and probably female B6C3F1 mice, causing a combination of hepatocellular carcinomas and hepatocellular adenomas. Nithiazide was also carcinogenic in female Fischer 344 rats, causing an increase in the incidence of mammary neoplasms. The compound was not carcinogenic in male Fischer 344 rats.

TABLE OF CONTENTS

Page

I. INTRODUCTION 1

II. MATERIALS AND METHODS 3

- A. Chemicals 3
- B. Dietary Preparation 5
- C. Animals 5
- D. Animal Maintenance 6
- E. Selection of Initial Concentrations 8
- F. Experimental Design 9
- G. Clinical and Histopathologic Examinations 12
- H. Data Recording and Statistical Analyses 14

III. CHRONIC TESTING RESULTS: RATS 19

- A. Body Weights and Clinical Observations 19
- B. Survival 19
- C. Pathology 19
- D. Statistical Analyses of Results 22

IV. CHRONIC TESTING RESULTS: MICE 30

- A. Body Weights and Clinical Observations 30
- B. Survival 30
- C. Pathology 34
- D. Statistical Analyses of Results 35

V. DISCUSSION 41

VI. BIBLIOGRAPHY 43

APPENDIX A SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH NITHIAZIDE A-1

APPENDIX B SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH NITHIAZIDE B-1

APPENDIX C SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH NITHIAZIDE C-1

APPENDIX D SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH NITHIAZIDE D-1

LIST OF ILLUSTRATIONS

Figure Number		Page
1	CHEMICAL STRUCTURE OF NITHIAZIDE	2
2	GROWTH CURVES FOR NITHIAZIDE CHRONIC STUDY RATS	20
3	SURVIVAL COMPARISONS OF NITHIAZIDE CHRONIC STUDY RATS	21
4	GROWTH CURVES FOR NITHIAZIDE CHRONIC STUDY MICE	31
5	SURVIVAL COMPARISONS OF NITHIAZIDE CHRONIC STUDY MICE	32
6	PERCENT SURVIVAL OF NITHIAZIDE CHRONIC STUDY MICE	33

LIST OF TABLES

Table Number		Page
1	DESIGN SUMMARY FOR FISCHER 344 RATS--NITHIAZIDE FEEDING EXPERIMENT	10
2	DESIGN SUMMARY FOR B6C3F1 MICE--NITHIAZIDE FEEDING EXPERIMENT	11
3	ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE RATS TREATED WITH NITHIAZIDE	23
4	ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE RATS TREATED WITH NITHIAZIDE	26
5	ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH NITHIAZIDE	36
6	ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH NITHIAZIDE	38

LIST OF TABLES (Concluded)

Table Number		Page
A1	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH NITHIAZIDE	A-3
A2	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH NITHIAZIDE	A-7
B1	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH NITHIAZIDE	B-3
B2	SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH NITHIAZIDE	B-7
C1	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH NITHIAZIDE	C-3
C2	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH NITHIAZIDE	C-8
D1	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH NITHIAZIDE	D-3
D2	SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH NITHIAZIDE	D-7

I. INTRODUCTION

Nithiazide (Figure 1) (NCI No. C03792), an antiprotozoal compound used in veterinary medicine, was selected for bioassay by the National Cancer Institute because of its use and possible persistence in the tissues and eggs of animals raised for human consumption.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is N-ethyl-N'-(5-nitro-2-thiazolyl) urea.[*] It is also called 1-ethyl-3-(5-nitro-2-thiazolyl) urea.

Nithiazide is most commonly used against *Histomonas maleagridas*, the organism which causes blackhead in fowl, particularly turkeys (O'Neill et al., 1956; Rose and Rose, 1966).

Specific production data for nithiazide are not available; however, the exclusion of this compound from *Synthetic Organic Chemicals: United States Production and Sales, 1976* (U.S. International Trade Commission, 1977) implies that nithiazide is not produced in commercial quantities (in excess of 1000 pounds or $1000 in value annually) in the United States.

The potential for exposure to nithiazide is greatest among veterinary workers and workers in facilities which produce this compound. The compound may persist in the tissues and eggs of treated poultry, thereby constituting a potential for more widespread human exposure.

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[*] The CAS registry number is 139-94-6.

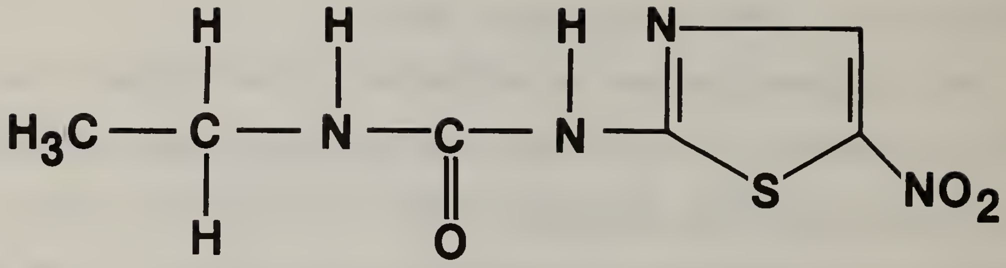

FIGURE 1
CHEMICAL STRUCTURE OF NITHIAZIDE

II. MATERIALS AND METHODS

A. Chemicals

Nithiazide was purchased from Merck, Sharp and Dohme Research Laboratory, a division of Merck and Company, Inc., Rahway, New Jersey. Chemical analysis was performed by Midwest Research Institute, Kansas City, Missouri. The range of the experimentally determined melting point (223° to 230°C) included the reported literature value of 228°C (O'Neill et al., 1956). Thin-layer chromatography (TLC) was performed utilizing two solvent systems (i.e., ethyl acetate:acetone and chloroform:methanol). Each plate was visualized with 354 and 367 nm light and the reducing agent p-nitroso-dimethylaniline. The plate developed with the first solvent system revealed one impurity, which remained at the origin, while the plate developed with the other solvent system indicated one motile and one nonmotile impurity. Elemental analysis was consistent with the molecular formula for nithiazide. High pressure liquid chromatography showed one homogenous peak. The results of infrared (IR) and nuclear magnetic resonance (NMR) analyses were consistent with those expected on a structural basis. Ultraviolet/visible (UV/VIS) analysis revealed λ_{max} at 233, 352 and 429 nm with respective molar extinction coefficients (ϵ) of 73.7×10^2, 13×10^3 and 19.8×10^2. The results are suggestive of a high purity compound.

A second batch of the compound was purchased from the same supplier. TLC was performed utilizing two solvent systems (i.e., ethyl acetate:acetone and benzene:1,4-dioxane). When visualized with ultraviolet light and p-nitroso-dimethylaniline/dimethylaminobenzaldehyde, each plate revealed one nonmotile impurity. High pressure liquid chromatography showed the presence of two minor and one major peak. Elemental analysis was within 5 percent of the theoretical. The range of the experimentally determined melting point (224° to 235°C) once again included the literature value (O'Neill et al., 1956). IR and NMR analyses were consistent with those expected based upon the structure of the compound. UV/VIS analysis showed λ_{max} of 233, 353, and 443 nm with ϵ values of 8.33×10^3, 11.8×10^3, and 5.3×10^3.

A third batch of the compound was purchased from the same supplier. TLC was performed utilizing two solvent systems (i.e., ethyl acetate:acetone and benzene:1,4-dioxane). When visualized with ultraviolet light and p-nitroso-dimethylaniline/dimethylaminobenzaldehyde, each plate revealed one impurity, remaining at the origin. High pressure liquid chromatography showed the presence of one homogeneous peak. Elemental analysis was within the acceptable limits of experimental variation (± 5 percent), based upon the molecular structure of the compound. The determined point of thermal decomposition was in general agreement with the point reported in the literature (O'Neill et al., 1956). IR and NMR analyses were consistent with the results expected based upon the structure of the compound. UV/VIS analysis showed λ_{max} of 232.5, 352, and 436 nm with ϵ values of 73.7×10^2, 13.1×10^3, and 6.75×10^2, respectively.

Throughout this report the term nithiazide is used to represent these compounds.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois). Nithiazide was administered to the dosed animals as a component of the diet.

The chemical was removed from its container and a proper amount was blended with an aliquot of the feed using a mortar and pestle. Once visual homogeneity was attained, the mixture was placed in a 6 kg capacity Patterson-Kelley standard model twin-shell stainless steel V-blender along with the remainder of the feed to be prepared. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. The mixture was prepared once weekly.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Rats were supplied by the Frederick Cancer Research Center, Frederick, Maryland. Mice were supplied by Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

Rats and mice were approximately 4 weeks old when received. Upon receipt, animals were examined for visible signs of disease or parasites. Obviously ill or runted animals were culled. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals which did not manifest clinical signs of disease were placed on test at this time. Animals were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

Animals were housed by species in rooms with a temperature range of 22° to 26°C and a range in relative humidity of 45 to 55 percent. Incoming air was filtered through HEPA filters (Flanders Filters, McLean, Virginia) at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided 8 hours per day (9:00 a.m. to 5:00 p.m.).

Rats were housed four per cage by sex and mice were housed five per cage by sex. Throughout the study dosed and control animals of both species were housed in polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) suspended from aluminum racks. Racks were fitted with a continuous stainless steel mesh lid over which a sheet of filter paper was firmly secured. Filter paper was changed at 2-week intervals, when the racks were sanitized. Clean cages and bedding were provided twice weekly. Ab-sorb-dri® hardwood chip bedding (Wilner Wood Products Company, Norway, Maine) was used in polycarbonate cages for the entire bioassay.

Acidulated water (pH 2.5) was supplied to animals in water bottles filled by an automated metering device that was checked daily for diluting accuracy. Water bottles were changed twice weekly and sipper tubes were washed at weekly intervals. During the period of chemical administration, dosed and control animals received treated or untreated Wayne Lab-Blox® meal as appropriate. The feed was supplied in hanging stainless steel hoppers which were refilled three times per week and sanitized weekly. Food and water were available _ad libitum_ for both species.

All dosed and control rats were housed in a room with other rats receiving diets containing* 2,4-dimethoxyaniline hydrochloride (54150-69-5) and 4'-(chloroacetyl)-acetanilide (140-49-8); and other rats intubated with dosed solutions of trimethylphosphate (512-56-1).

All dosed and control mice were housed in a room with other mice receiving diets containing 2,4-dimethoxyaniline hydrochloride (54150-69-5); 4'-(chloroacetyl)-acetanilide (140-49-8); p-phenylenediamine dihydrochloride (624-18-0); 4-nitro-o-phenylenediamine (99-56-9); and 1-phenyl-3-methyl-5-pyrazolone (89-25-8); and other mice intubated with dosed solutions of trimethylphosphate (512-56-1); 2-(chloromethyl)pyridine hydrochloride (6959-47-3); 3-(chloromethyl) pyridine hydrochloride (3099-31-8); and pivalolactone (1955-45-9).

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*CAS registry numbers are given in parentheses.

E. Selection of Initial Concentrations

In order to establish the concentrations of nithiazide for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Rats were distributed among thirteen groups, each consisting of five males and five females. Nithiazide was incorporated into the basal laboratory diet and supplied *ad libitum* to eleven of the thirteen rat groups in concentrations of 464, 681, 1000, 1470, 2150, 3160, 4640, 6810, 10,000, 14,700 and 21,500 ppm. The two remaining rat groups served as control groups, receiving only the basal laboratory diet.

Mice were distributed among six groups, each consisting of five males and five females. Nithiazide was incorporated into the basal laboratory diet and supplied *ad libitum* to five of the six mouse groups in concentrations of 6800, 10,000, 14,700, 21,600 and 31,500 ppm. The sixth mouse group served as a control group, receiving only the basal laboratory diet.

The dosed dietary preparations were administered for a period of 7 weeks, followed by a 1-week observation period during which all animals were fed the basal laboratory diet. Individual body weights and food consumption data were recorded twice weekly throughout the study. Upon termination of the observation period, all survivors were sacrificed and necropsied.

At 21,500 ppm, one male rat died, while all female rats receiving the same concentration died. At the end of the subchronic test, the mean body weight gain of both male and female rats dosed with 1470 ppm was 21 percent less than the mean body weight gain of their respective controls. At a dietary concentration of 1000 ppm, the mean body weight gain of male rats was 12 percent less than that of their controls, while female rats receiving the same concentration displayed a mean body weight gain 12 percent less than that of their controls. At both of these concentrations yellow patches on the coat were observed. The high concentration selected for administration to dosed rats in the chronic bioassay was 1250 ppm.

At a dietary concentration of 31,500 ppm, one male mouse died. Two female mice died at a concentration of 21,600 ppm. At the end of the subchronic test, the mean body weight gain of both male and female mice dosed with 6800 ppm was 10 percent less than the mean body weight gain of their respective controls. The high concentration selected for administration to dosed mice in the chronic bioassay was 5000 ppm.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, concentrations administered, and duration of treated and untreated observation periods) are summarized in Tables 1 and 2.

All rats were approximately 6 weeks old at the time the test was initiated and were placed on test simultaneously. The dietary concentrations of nithiazide administered were 1250 and 625 ppm.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS NITHIAZIDE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	NITHIAZIDE CONCENTRATION[a]	OBSERVATION PERIOD	
			TREATED (WEEKS)	UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	104
LOW DOSE	50	625	38	
		0		9
		625	56	
		0		1
HIGH DOSE	50	1250	38	
		0		9
		1250	56	
		0		1
FEMALE				
CONTROL	20	0	0	104
LOW DOSE	50	625	38	
		0		9
		625	56	
		0		1
HIGH DOSE	50	1250	38	
		0		9
		1250	56	
		0		1

[a] Concentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE NITHIAZIDE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	NITHIAZIDE CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	104
LOW DOSE	50	2500	61	
		0		9
		2500	33	
		0		1
HIGH DOSE	50	5000	61	
		0		9
		5000	33	
		0		1
FEMALE				
CONTROL	20	0	0	105
LOW DOSE	50	2500	61	
		0		9
		2500	33	
		0		1
HIGH DOSE	50	5000	61	
		0		9
		5000	33	
		0		1

[a] Concentrations given in parts per million.

Throughout this report, those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. Dosed rats were supplied with feed containing nithiazide for the first 38 weeks of the chronic study. Due to a shortage of nithiazide, dosed diets were not available for the following 9-week period. Use of dosed feed was then resumed and continued for 56 weeks, followed by a 1-week observation period.

All mice were approximately 6 weeks old at the time the test was initiated, and were placed on test simultaneously. The dietary concentrations of nithiazide administered were 5000 and 2500 ppm. Throughout this report, those mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. Dosed mice were supplied with feed containing nithiazide for the first 61 weeks of the chronic study. Due to a shortage of nithiazide, dosed diets were not available for the following 9-week period. Use of dosed feed was then resumed and continued for 33 weeks, followed by a 1-week observation period for males, and a 2-week observation period for females.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. From the first day, all animals were inspected twice daily for mortality. Food consumption data were collected at monthly intervals from 20 percent of the animals in each group. Body weights were recorded once monthly throughout this bioassay.

All moribund animals or animals that developed large, palpable masses that jeopardized their health were killed. A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was killed at the end of the bioassay. The animals were euthanized using carbon dioxide, and were immediately necropsied. Gross and microscopic examinations were performed on all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in a 10 percent neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, tunica vaginalis, uterus, mammary gland, and ovary.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were recorded in each group at the time that the test was initiated.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

For both male and female rats there was slight, although distinct, dose-related mean body weight depression (Figure 2).

No unusual clinical observations were reported.

B. Survival

The estimated probabilities of survival for male and female rats in the control and nithiazide-dosed groups are shown in Figure 3. For both males and females, the Tarone test for positive association between dosage and mortality was not significant.

There were adequate numbers of male rats at risk from late-developing tumors, as 33/50 (66 percent) of the high dose, 32/50 (64 percent) of the low dose and 16/20 (80 percent) of the control group survived on test until termination of the study. For female rats, with 42/50 (84 percent) of the high dose, 33/50 (66 percent) of the low dose, and 17/20 (85 percent) of the control group surviving on test until the termination of the study, there were adequate numbers at risk from late-developing tumors.

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are summarized in Appendix C (Tables C1 and C2).

There was a variety of tumors in both the control and dosed groups. Some types of neoplasms occurred with greater frequency in

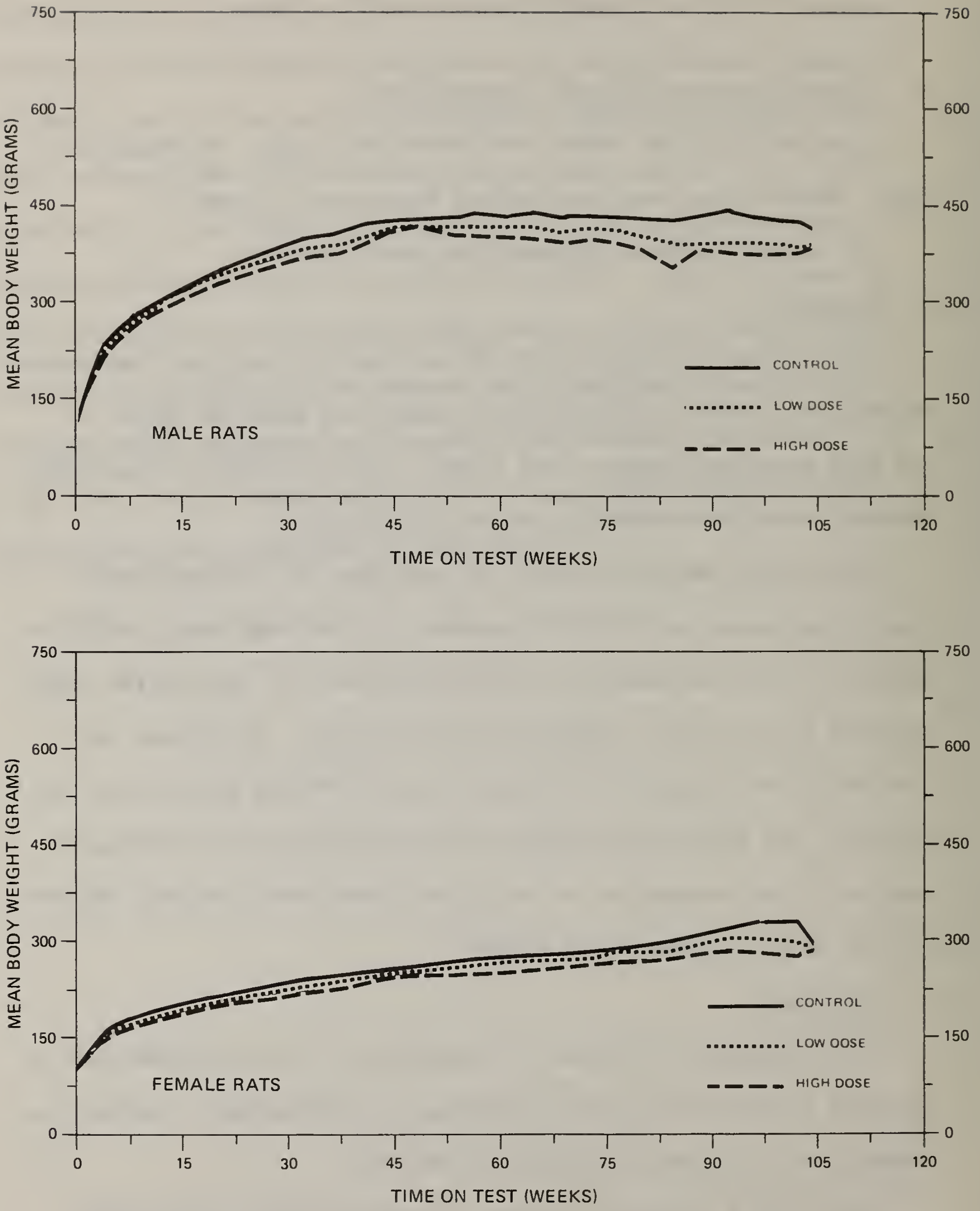
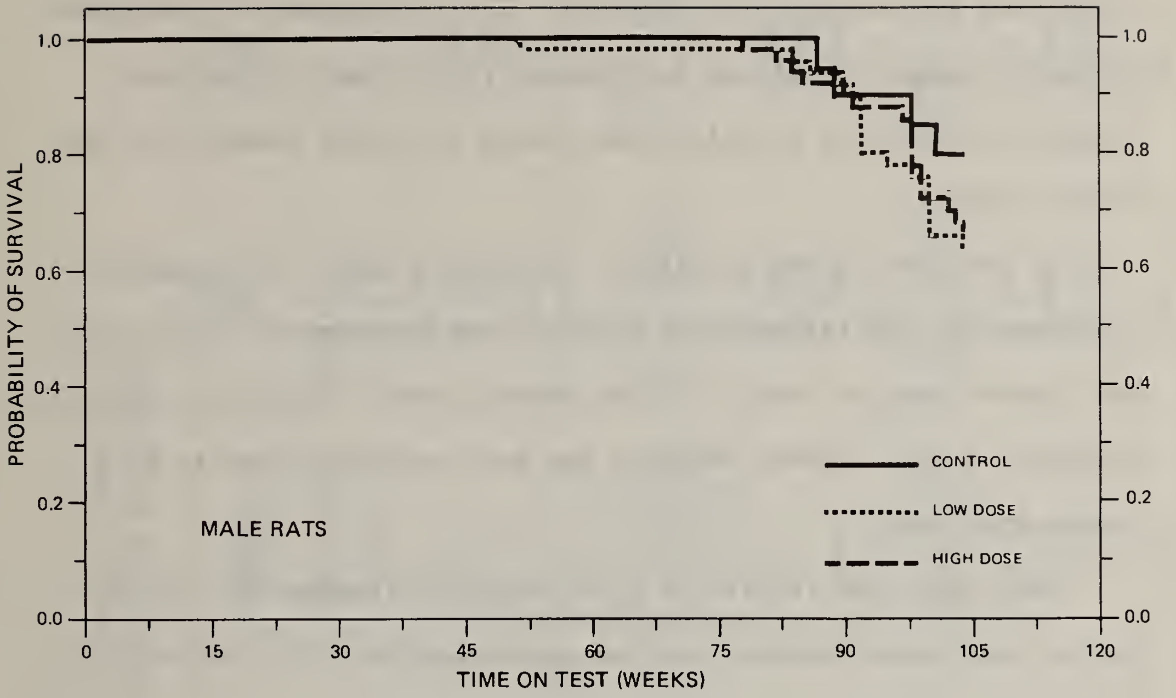


FIGURE 2
GROWTH CURVES FOR NITHIAZIDE CHRONIC STUDY RATS



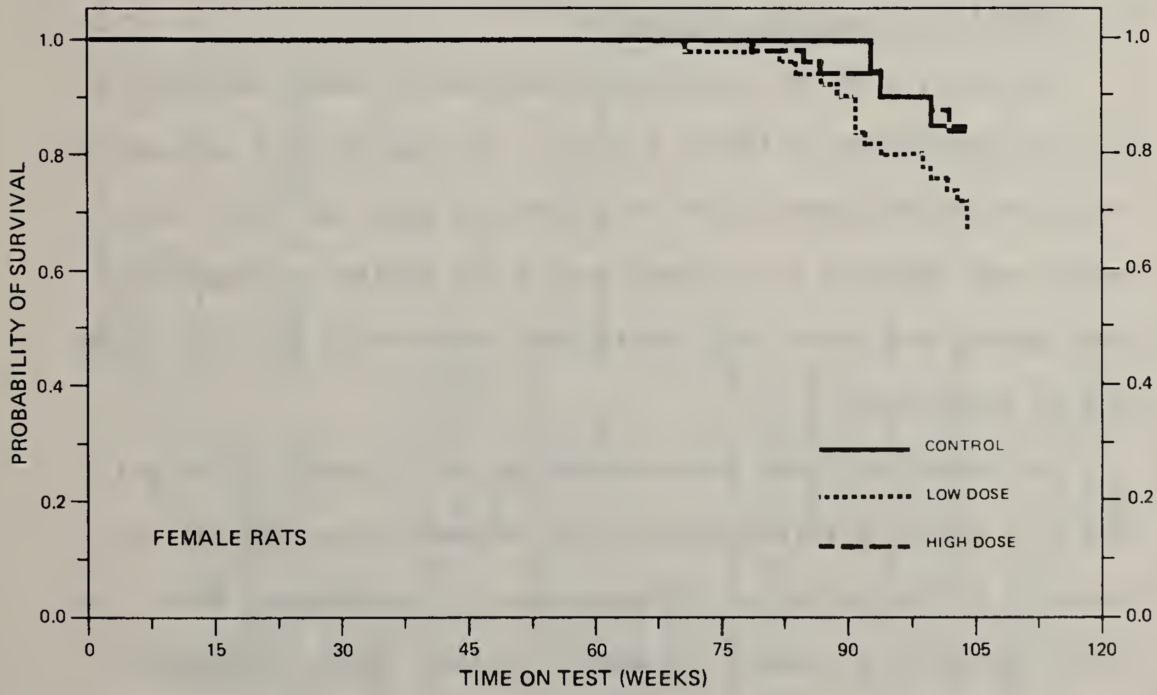


FIGURE 3
SURVIVAL COMPARISONS OF NITHIAZIDE CHRONIC STUDY RATS

dosed rats as compared with controls. The incidences of chromophobe adenomas, mammary neoplasms and endometrial stromal polyps were slightly elevated in the high dose female rats when compared to the female controls.

In addition to the neoplastic lesions, a number of degenerative, proliferative and inflammatory changes were encountered in the dosed and control groups. Most of these nonneoplastic lesions are commonly observed in aged Fischer 344 rats and were not considered to be compound-related.

Based upon the results of this pathology examination, there was no conclusive evidence for the carcinogenicity of nithiazide in Fischer 344 rats under the conditions of this study.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or nithiazide-dosed groups and where such tumors were observed in at least 5 percent of the group.

For female rats the Cochran-Armitage test indicated a significant ($P = 0.003$) positive association between dosage and the incidences of a combination of fibroadenomas or cystadenomas NOS of the skin, subcutaneous tissue, and mammary gland. This was supported

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE RATS TREATED WITH NITHIAZIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/20(0.20)	8/50(0.16)	15/50(0.30)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.800	1.500
Lower Limit	---	0.250	0.566
Upper Limit	---	3.327	5.627
Weeks to First Observed Tumor	87	90	78
Pituitary: Chromophobe Adenoma or Acidophil Adenoma[b]	6/15(0.40)	2/36(0.06)	2/30(0.07)
P Values[c]	P = 0.007(N)	P = 0.005(N)	P = 0.011(N)
Departure from Linear Trend[e]	P = 0.018	---	---
Relative Risk (Control)[d]	---	0.139	0.167
Lower Limit	---	0.016	0.020
Upper Limit	---	0.685	0.814
Weeks to First Observed Tumor	104	95	85
Adrenal: Pheochromocytoma[b]	2/20(0.10)	2/50(0.04)	6/50(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.400	1.200
Lower Limit	---	0.032	0.243
Upper Limit	---	5.277	11.574
Weeks to First Observed Tumor	104	104	98

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pancreatic Islets: Islet-Cell Adenoma or Islet-Cell Carcinoma[b]	1/19(0.05)	3/50(0.06)	0/46(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.140	0.000
Lower Limit	---	0.101	0.000
Upper Limit	---	58.635	7.707
Weeks to First Observed Tumor	104	100	---
Testis: Interstitial-Cell Tumor[b]	19/20(0.95)	47/50(0.94)	48/50(0.96)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.989	1.011
Lower Limit	---	0.922	0.942
Upper Limit	---	1.168	1.149
Weeks to First Observed Tumor	87	86	78
Body Cavities: Mesothelioma NOS or Malignant Mesothelioma[b]	0/20(0.00)	4/50(0.08)	0/50(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.021	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.386	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	52	---

TABLE 3 (CONCLUDED)

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[a] Treated groups received doses of 625 or 1250 ppm in feed.

[b] Number of tumor-bearing animals/number of animals examined at site (proportion).

[c] The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d] The 95% confidence interval on the relative risk of the treated group to the control group.

[e] The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE RATS TREATED WITH NITHIAZIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Skin, Subcutaneous Tissue, and Mammary Gland: Fibroadenoma or Cystadenoma NOS[b]	1/20(0.05)	5/50(0.10)	15/50(0.30)
P Values[c]	P = 0.003	N.S.	P = 0.020
Relative Risk (Control)[d]	---	2.000	6.000
Lower Limit	---	0.249	1.048
Upper Limit	---	92.596	245.704
Weeks to First Observed Tumor	93	89	94
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	3/20(0.15)	9/50(0.18)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.200	0.400
Lower Limit	---	0.346	0.060
Upper Limit	---	6.408	2.802
Weeks to First Observed Tumor	94	71	79
Pituitary: Chromophobe Adenoma or Acidophil Adenoma[b]	5/18(0.28)	13/39(0.33)	24/47(0.51)
P Values[c]	P = 0.034	N.S.	N.S.
Relative Risk (Control)[d]	---	1.200	1.838
Lower Limit	---	0.493	0.852
Upper Limit	---	3.750	5.313
Weeks to First Observed Tumor	93	89	94

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Adenoma[b]	2/19(0.11)	1/38(0.03)	0/26(0.00)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.250	0.000
Lower Limit	---	0.004	0.000
Upper Limit	---	4.557	2.404
Weeks to First Observed Tumor	104	104	---
Pancreatic Islets: Islet-Cell Adenoma or Islet-Cell Carcinoma[b]	0/19(0.00)	0/48(0.00)	3/47(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.254
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	100
Uterus: Endometrial Stromal Polyp[b]	1/19(0.05)	4/50(0.08)	10/50(0.20)
P Values[c]	$P = 0.039$	N.S.	N.S.
Relative Risk (Control)[d]	---	1.520	3.800
Lower Limit	---	0.167	0.613
Upper Limit	---	73.309	160.949
Weeks to First Observed Tumor	104	91	87

TABLE 4 (CONCLUDED)

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[a] Treated groups received doses of 625 or 1250 ppm in feed.

[b] Number of tumor-bearing animals/number of animals examined at site (proportion).

[c] The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d] The 95% confidence interval on the relative risk of the treated group to the control group.

by a significant (P = 0.020) positive Fisher exact test comparing the high dose group to the control group.

Based on these statistical results, nithiazide was carcinogenic in female Fischer 344 rats under the conditions of this bioassay.

In female rats the Cochran-Armitage test for association between dosage and incidence was significant for a combination of chromophobe adenomas and acidophil adenomas of the pituitary (P = 0.034) and also for endometrial stromal polyps of the uterus (P = 0.039). However, in both cases, the Fisher exact tests comparing the high dose group to the control and the low dose group to the control were not significant.

None of the statistical tests for any site in male rats indicated a significant positive association between the administration of nithiazide and an increased tumor incidence.

The possibility of a negative association between dose and tumor incidence was noted in male rats for chromophobe adenomas or acidophil adenomas of the pituitary. The historical incidence of these tumors in untreated male Fischer 344 rats from control data collected by this laboratory for the NCI Carcinogenesis Testing Program was 23/188 (12 percent) as compared with the 6/15 (40 percent) incidence in the controls of this bioassay.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

For both male and female mice there was a distinct and consistent dose-related mean body weight depression (Figure 4).

No unusual clinical observations were reported.

B. Survival

The estimated probabilities of survival for male and female mice in the control and nithiazide-dosed groups are shown in Figure 5. For both males and females the Tarone test for association between dosage and mortality was not significant.

The percentages of male and female mice surviving on test are shown in Figure 6. Although 6 high dose and 3 low dose male mice were missing by week 12, adequate numbers of males were at risk from late-developing tumors as 39/50 (78 percent) of the high dose, 36/50 (72 percent) of the low dose and 15/20 (75 percent) of the control group survived on test until the termination of the study.

There were also adequate numbers of female mice at risk from late-developing tumors. Although 4 high dose, 8 low dose, and 1 control female mice were missing by week 12, 39/50 (78 percent) of the high dose, 39/50 (78 percent) of the low dose, and 17/20 (85 percent) of the control group survived on test until the termination of the study.

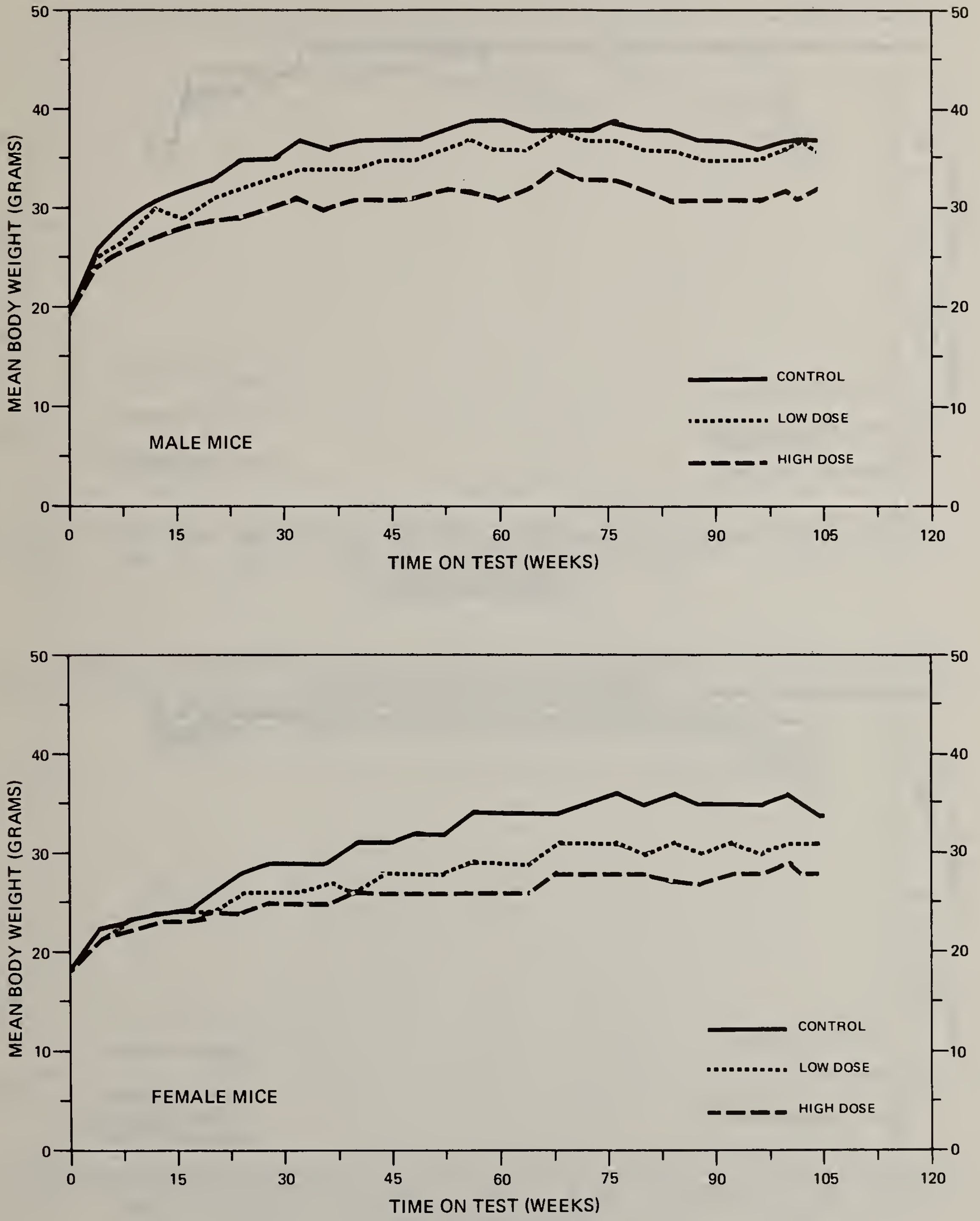
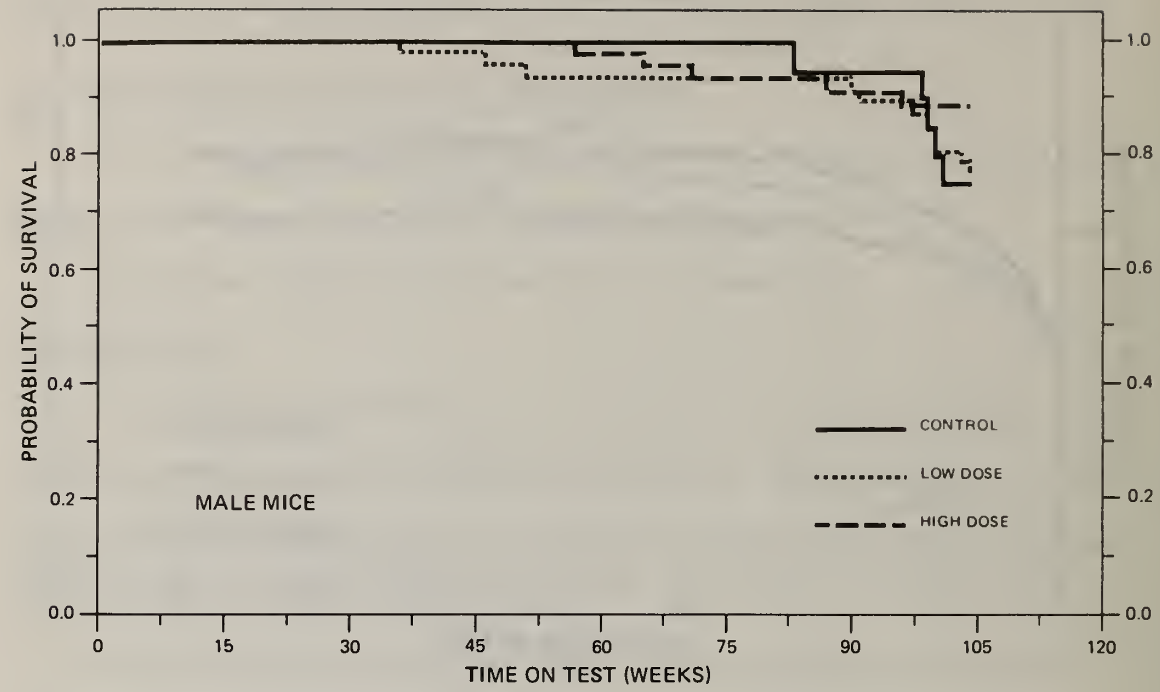


FIGURE 4
GROWTH CURVES FOR NITHIAZIDE CHRONIC STUDY MICE



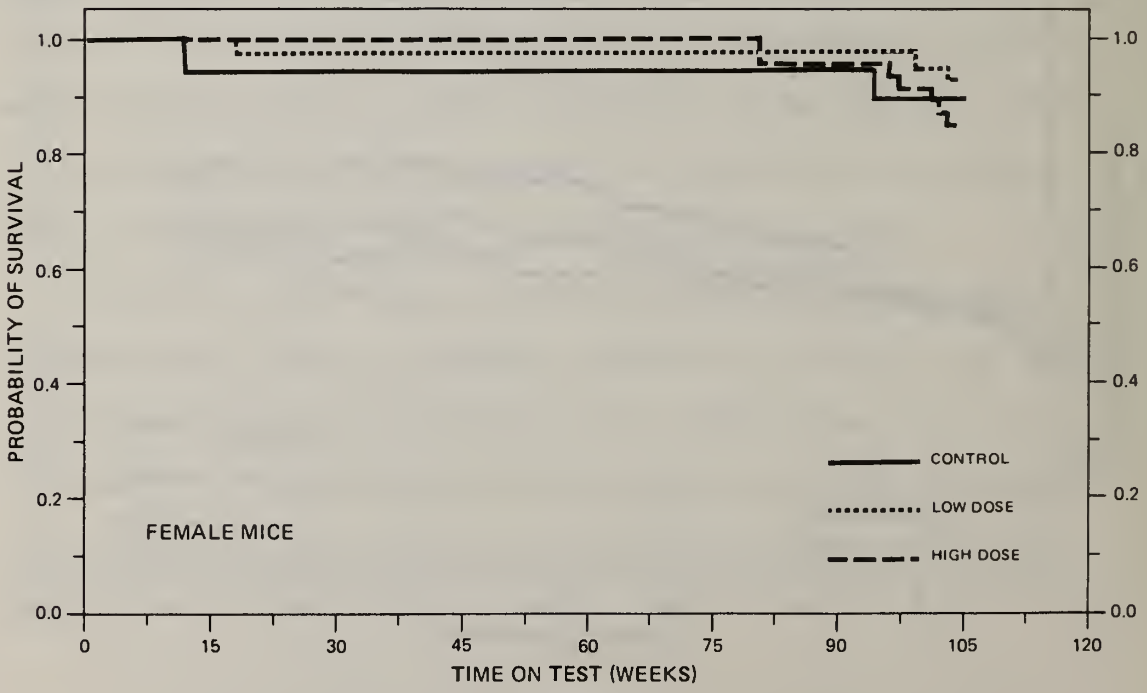


FIGURE 5
SURVIVAL PROBABILITY COMPARISONS OF NITHIAZIDE CHRONIC STUDY MICE

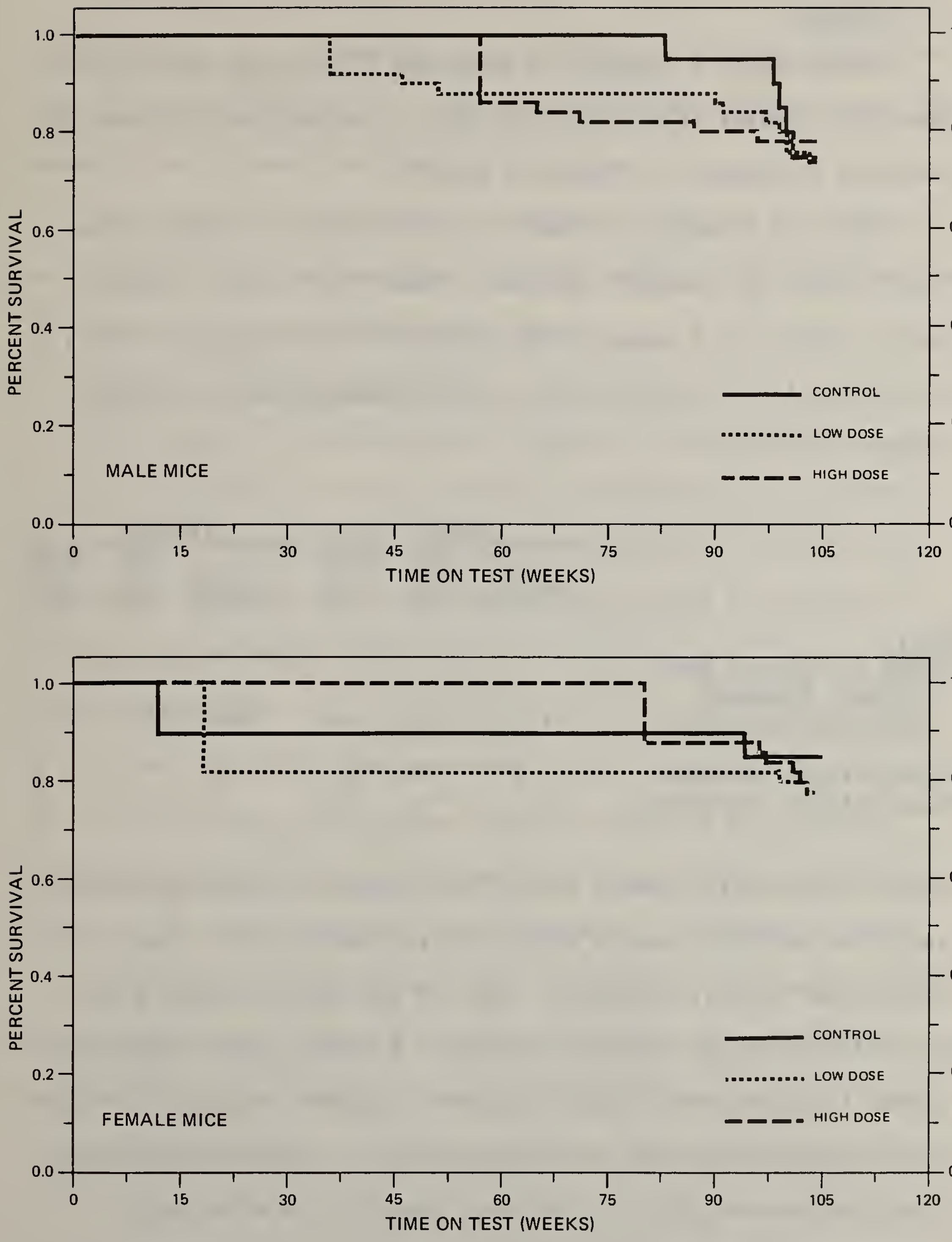


FIGURE 6
PERCENT SURVIVAL OF NITHIAZIDE CHRONIC STUDY MICE

C. Pathology

Histopathologic findings on neoplasms in mice are tabulated in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are tabulated in Appendix D (Tables D1 and D2).

There was a variety of tumors in the control and dosed groups. These lesions are commonly observed in aged B6C3F1 mice. Hepatic tumors, however, did appear to be elevated in the high dose groups when compared to controls, particularly among male mice as shown below:

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Liver						
Number of Animals with Tissues Examined Histopathologically	(20)	(46)	(43)	(18)	(41)	(43)
Hepatocellular Adenoma	2	9	13	2	4	8
Hepatocellular Carcinoma	2	6	12	1	0	4

Grossly, the hepatic tumors varied from inapparent single or multiple lesions to nodules, ranging up to 3 cm in diameter. The larger lesions were usually malignant. Most of the hepatic tumors were well-differentiated, expanding lesions. A spindle-cell component was present in one hepatocellular carcinoma. Criteria employed to determine malignancy included: metastasis, local invasion, formation of trabecular pattern, mitotic activity, anaplasia, and necrosis.

In addition to the neoplastic lesions a number of degenerative, proliferative, and inflammatory changes were encountered in dosed and control groups (Appendix C). Most of these nonneoplastic lesions are commonly seen in aged laboratory B6C3F1 mice.

The results of this pathology examination suggest that nithiazide was responsible for the observed increased number of hepatic tumors in the high dose male and female B6C3F1 mice.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or nithiazide-dosed groups and where such tumors were observed in at least 5 percent of the group.

For male mice the Cochran-Armitage test indicated a significant ($P = 0.002$) positive association between dosage and the combined incidence of hepatocellular carcinomas or hepatocellular adenomas. Additionally, the Fisher exact test comparing the high dose group to the control group was significant ($P = 0.005$). In female mice an unusually large, though not statistically significant, number of hepatocellular carcinomas or hepatocellular adenomas was also found in the high dose group. It should be noted that in historical control data collected by this laboratory for the NCI Carcinogenesis Testing Program 9/207 (4 percent) untreated female B6C3F1 mice had

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH NITHIAZIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	3/20(0.15)	4/44(0.09)	1/44(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.606	0.152
Lower Limit	---	0.116	0.003
Upper Limit	---	3.855	1.774
Weeks to First Observed Tumor	99	104	104
Hematopoietic System: Malignant Lymphoma[b]	4/20(0.20)	6/47(0.13)	4/44(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.638	0.455
Lower Limit	---	0.175	0.096
Upper Limit	---	2.820	2.242
Weeks to First Observed Tumor	98	90	104
Liver: Hepatocellular Carcinoma[b]	2/20(0.10)	6/46(0.13)	12/43(0.28)
P Values[c]	$P = 0.037$	N.S.	N.S.
Relative Risk (Control)[d]	---	1.304	2.791
Lower Limit	---	0.264	0.717
Upper Limit	---	12.541	24.104
Weeks to First Observed Tumor	100	104	104

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	4/20(0.20)	15/46(0.33)	25/43(0.58)
P Values[c]	P = 0.002	N.S.	P = 0.005
Relative Risk (Control)[d]	---	1.630	2.907
Lower Limit	---	0.617	1.217
Upper Limit	---	6.077	9.903
Weeks to First Observed Tumor	99	104	87
Adrenal: Pheochromocytoma[b]	2/17(0.12)	0/42(0.00)	0/41(0.00)
P Values[c]	P = 0.026(N)	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.045	---	---
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	1.353	1.385
Weeks to First Observed Tumor	104	---	---

[a] Treated groups received doses of 2500 or 5000 ppm in feed.

[b] Number of tumor-bearing animals/number of animals examined at site (proportion).

[c] The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d] The 95% confidence interval on the relative risk of the treated group to the control group.

[e] The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH NITHIAZIDE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	3/16(0.19)	1/42(0.02)	2/43(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.127	0.248
Lower Limit	---	0.003	0.023
Upper Limit	---	1.474	2.011
Weeks to First Observed Tumor	104	104	104
Hematopoietic System: Malignant Lymphoma[b]	3/18(0.17)	7/42(0.17)	8/45(0.18)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.000	1.067
Lower Limit	---	0.267	0.300
Upper Limit	---	5.520	5.761
Weeks to First Observed Tumor	94	99	80
Liver: Hepatocellular Carcinoma[b]	1/18(0.06)	0/41(0.00)	4/43(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	1.674
Lower Limit	---	0.000	0.186
Upper Limit	---	8.171	80.455
Weeks to First Observed Tumor	104	---	104

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	3/18(0.17)	4/41(0.10)	12/43(0.28)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.585	1.674
Lower Limit	---	0.114	0.536
Upper Limit	---	3.698	8.451
Weeks to First Observed Tumor	104	104	97

---

[a] Treated groups received doses of 2500 or 5000 ppm in feed.

[b] Number of tumor-bearing animals/number of animals examined at site (proportion).

[c] The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated groups(s) than in the control group.

[d] The 95% confidence interval on the relative risk of the treated group to the control group.

hepatocellular carcinomas or hepatocellular adenomas as compared with the 3/18 (17 percent) combined incidence found in the control group of this bioassay. Out of 11 female historical control groups observed, the incidence for the small control group in this bioassay was the highest. Based upon these statistical results the administration of nithiazide was associated with the increased incidence of liver neoplasms in male mice and possibly female mice under the conditions of this bioassay.

The Cochran-Armitage test indicated a significant negative association between administration and the incidence of adrenal pheochromocytomas in male mice. The Fisher exact tests, however, were not significant.

None of the statistical tests for any site in female mice indicated a significant positive association between the administration of nithiazide and an increased tumor incidence.

V. DISCUSSION

There were no significant positive associations between the concentrations of nithiazide administered and mortality in either species. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Slight dose-related mean body weight depression was observed in both male and female rats. Distinct and consistent dose-related mean body weight depression occurred in male and female mice.

Among female rats there was a significant positive association between dosage and the incidences of a combination of fibroadenomas and cystadenomas NOS of the skin, subcutaneous tissue, or mammary gland. This finding was supported by a significant high dose to control Fisher exact comparison. No other tumors occurred in statistically significant increased incidences when dosed female rats were compared to controls. None of the statistical tests indicated a positive association between the administration of nithiazide and increased tumor incidence in male rats.

In female mice, none of the statistical tests showed a significant positive association between increased tumor incidence and the administration of nithiazide. For male mice the combined incidence of hepatocellular carcinomas and hepatocellular adenomas (i.e., 4/20 in the controls, 15/46 in the low dose, and 25/43 in the high dose) was significantly dose-related and the combined incidence of these tumors in the high dose group was statistically significant when compared to the incidence in the male mouse control group. Furthermore almost half of the hepatocellular neoplasms observed in the dosed groups were carcinomas (i.e., 2/20, 6/46, and 12/43 in the control, low dose and high dose groups, respectively).

In female mice an unusually large, though not statistically significant, number of hepatocellular carcinomas or hepatocellular adenomas was also found in the high dose group. It should be noted that in historical data collected by this laboratory for the NCI Carcinogenesis Testing Program control female mice had 9/207 (4 percent) heptocellular carcinomas or hepatocellular adenomas as compared with the 3/18 (17 percent) combined incidence found in the control group of this bioassay. Out of 11 female historical control groups observed, the incidence for the control group in this bioassay was the highest. Although the increased incidences of these tumors in dosed female mice were not statistically significant, the evidence was strongly suggestive of carcinogenicity to the liver in female B6C3F1 mice.

Under the conditions of this bioassay, nithiazide was carcinogenic in male and probably in female B6C3F1 mice, causing a combination of hepatocellular carcinomas and hepatocellular adenomas. Nithiazide was also carcinogenic in female Fischer 344 rats, causing an increased incidence of a combination of skin and mammary neoplasms. The compound was not carcinogenic in male Fischer 344 rats.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH NITHIAZIDE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH NITHIAZIDE

	CONTROL(UNTR) 11-1345	LOW DOSE 11-1343	HIGH DOSE 11-1341
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SEBACEOUS ADENOMA			1 (2%)
KERATOACANTHOMA		1 (2%)	
FIBROMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ISLET-CELL CARCINOMA, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#BRAIN	(20)	(50)	(50)
MALIGNANT RETICULOSIS		1 (2%)	1 (2%)
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
LEUKEMIA,NOS	4 (20%)	6 (12%)	11 (22%)
UNDIFFERENTIATED LEUKEMIA			1 (2%)
CIRCULATORY SYSTEM			
NONE			

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1345	LOW DOSE 11-1343	HIGH DOSE 11-1341
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(49)
NEOPLASTIC NODULE		1 (2%)	2 (4%)
#SMALL INTESTINE	(20)	(49)	(49)
NEUROFIBROSARCOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(15)	(36)	(30)
CHROMOPHOBE ADENOMA	5 (33%)	2 (6%)	2 (7%)
ACIDOPHIL ADENOMA	1 (7%)		
#ADRENAL	(20)	(50)	(50)
PHEOCHROMOCYTOMA	2 (10%)	2 (4%)	6 (12%)
#THYROID	(15)	(30)	(21)
FOLLICULAR-CELL ADENOMA			1 (5%)
C-CELL ADENOMA	1 (7%)	1 (3%)	
#PARATHYROID	(6)	(16)	(11)
ADENOMA, NOS			1 (9%)
#PANCREATIC ISLETS	(19)	(50)	(46)
ISLET-CELL ADENOMA	1 (5%)	2 (4%)	
ISLET-CELL CARCINOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
FIBROADENOMA	1 (5%)	1 (2%)	1 (2%)
*PREPUTIAL GLAND	(20)	(50)	(50)
SEBACEOUS ADENOMA			1 (2%)
#TESTIS	(20)	(50)	(50)
INTERSTITIAL-CELL TUMOR	19 (95%)	47 (94%)	48 (96%)

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONTINUED)

	CONTROL(UNTR) 11-1345	LOW DOSE 11-1343	HIGH DOSE 11-1341
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*TARSAL GLAND	(20)	(50)	(50)
SEBACEOUS ADENOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*BODY CAVITIES	(20)	(50)	(50)
MESOTHELIOMA, MALIGNANT		1 (2%)	
*PERITONEUM	(20)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	
MESOTHELIOMA, MALIGNANT		1 (2%)	
*PERITONEAL CAVITY	(20)	(50)	(50)
SARCOMA, NOS		1 (2%)	
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
OSTEOSARCOMA			1 (2%)
THORACIC CAVITY			
SARCOMA, NOS		1	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 11-1345	LOW DOSE 11-1343	HIGH DOSE 11-1341
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	4	10
MORIBUND SACRIFICE	2	14	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	32	33
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	CONTROL (UNTR) 11-1345	LOW DOSE 11-1343	HIGH DOSE 11-1341
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	50	50
TOTAL PRIMARY TUMORS	34	78	82
TOTAL ANIMALS WITH BENIGN TUMORS	20	49	49
TOTAL BENIGN TUMORS	30	61	62
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	14	16
TOTAL MALIGNANT TUMORS	4	14	18
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT		3	2
TOTAL UNCERTAIN TUMORS		3	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH NITHIAZIDE

	CONTROL (UNTR) 11-1346	LOW DOSE 11-1344	HIGH DOSE 11-1342
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA, INVASI		1 (2%)	
FIBROMA		2 (4%)	
FIBROADENOMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
SARCOMA, NOS	1 (5%)		
FIBROADENOMA			4 (8%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LEUKEMIA,NOS	3 (15%)	9 (18%)	2 (4%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
TRANSITIONAL-CELL CARCINOMA	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1346	LOW DOSE 11-1344	HIGH DOSE 11-1342
#URINARY BLADDER	(14)	(37)	(37)
TRANSITIONAL-CELL PAPILLOMA			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(39)	(47)
CHROMOPHOBE ADENOMA	5 (28%)	13 (33%)	23 (49%)
ACIDOPHIL ADENOMA			1 (2%)
#ADRENAL	(20)	(50)	(48)
CORTICAL ADENOMA			1 (2%)
#THYROID	(19)	(38)	(26)
C-CELL ADENOMA	2 (11%)	1 (3%)	
#PANCREATIC ISLETS	(19)	(48)	(47)
ISLET-CELL ADENOMA			1 (2%)
ISLET-CELL CARCINOMA			2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CYSTADENOMA, NOS		1 (2%)	2 (4%)
FIBROADENOMA	1 (5%)	4 (8%)	8 (16%)
#UTERUS	(19)	(50)	(50)
ENDOMETRIAL STROMAL POLYP	1 (5%)	4 (8%)	10 (20%)
HEMANGIOMA	1 (5%)		
#CERVIX UTERI	(19)	(50)	(50)
SARCOMA, NOS	1 (5%)		
HEMANGIOSARCOMA		1 (2%)	
#UTERUS/ENDOMETRIUM	(19)	(50)	(50)
ADENOCARCINOMA, NOS		2 (4%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1346	LOW DOSE 11-1344	HIGH DOSE 11-1342
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	11	3
MORIBUND SACRIFICE	1	6	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	33	42
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 11-1346	LOW DOSE 11-1344	HIGH DOSE 11-1342
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	28	38
TOTAL PRIMARY TUMORS	16	38	57
TOTAL ANIMALS WITH BENIGN TUMORS	7	22	37
TOTAL BENIGN TUMORS	10	25	52
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	12	5
TOTAL MALIGNANT TUMORS	6	13	5
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH NITHIAZIDE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH NITHIAZIDE

	CONTROL (UNTR) 22-2345	LOW DOSE 22-2343	HIGH DOSE 22-2341
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		3	6
ANIMALS NECROPSIED	20	47	44
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	47	44
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(47)	(44)
NEUROFIBROMA			1 (2%)
*SUBCUT TISSUE	(20)	(47)	(44)
NEUROFIBROMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(44)	(44)
NEOPLASM, NOS, METASTATIC		1 (2%)	
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (15%)	4 (9%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(47)	(44)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (5%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	1 (2%)
*ABDOMINAL CAVITY	(20)	(47)	(44)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		
#SPLEEN	(19)	(46)	(43)
NEOPLASM, NOS, METASTATIC		1 (2%)	
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#MESENTERIC L. NODE	(19)	(43)	(43)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	1 (2%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2345	LOW DOSE 22-2343	HIGH DOSE 22-2341
#HEART	(19)	(42)	(42)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#SMALL INTESTINE	(19)	(44)	(43)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(46)	(43)
NEOPLASM, NOS, METASTATIC		1 (2%)	
HEPATOCELLULAR ADENOMA	2 (10%)	9 (20%)	13 (30%)
HEPATOCELLULAR CARCINOMA	2 (10%)	6 (13%)	12 (28%)
URINARY SYSTEM			
#KIDNEY	(19)	(46)	(42)
NEOPLASM, NOS, METASTATIC		1 (2%)	
#URINARY BLADDER	(16)	(36)	(40)
HEMANGIOMA		1 (3%)	
ENDOCRINE SYSTEM			
#ADRENAL	(17)	(42)	(41)
NEOPLASM, NOS, MALIGNANT		1 (2%)	
CORTICAL ADENOMA		1 (2%)	
PHEOCHROMOCYTOMA	2 (12%)		
#THYROID	(14)	(29)	(31)
FOLLICULAR-CELL ADENOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(18)	(44)	(42)
INTERSTITIAL-CELL TUMOR			1 (2%)
NERVOUS SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2345	LOW DOSE 22-2343	HIGH DOSE 22-2341
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(20)	(47)	(44)
MESOTHELIOMA, MALIGNANT		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	5	10	4
MORIBUND SACRIFICE		1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	36	39
ANIMAL MISSING		3	6

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 22-2345	LOW DOSE 22-2343	HIGH DOSE 22-2341
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	10	27	28
TOTAL PRIMARY TUMORS	13	31	32
TOTAL ANIMALS WITH BENIGN TUMORS	5	16	15
TOTAL BENIGN TUMORS	7	17	16
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	14	16
TOTAL MALIGNANT TUMORS	6	14	16
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	
TOTAL SECONDARY TUMORS	1	4	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH NITHIAZIDE

	CONTROL(UNTR) 22-2346	LOW DOSE 22-2344	HIGH DOSE 22-2342
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	2	8	4
ANIMALS NECROPSIED	18	42	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	18	42	45
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(18)	(42)	(45)
FIBROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(16)	(42)	(43)
HEPATOCELLULAR CARCINOMA, METAST	1 (6%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (19%)	1 (2%)	2 (5%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(18)	(42)	(45)
MALIGNANT LYMPHOMA, NOS	1 (6%)	2 (5%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	2 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (6%)	1 (2%)	1 (2%)
*ABDOMINAL WALL	(18)	(42)	(45)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#MESENTERIC L. NODE	(18)	(38)	(40)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)
#HEART	(18)	(36)	(40)
MALIGNANT LYMPHOMA, NOS		1 (3%)	
#LIVER	(18)	(41)	(43)
MALIGNANT LYMPHOMA, NOS	1 (6%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			2 (5%)
KUPFFER-CELL SARCOMA			1 (2%)
#SMALL INTESTINE	(18)	(40)	(44)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (3%)	

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2346	LOW DOSE 22-2344	HIGH DOSE 22-2342
#COLON	(18)	(38)	(43)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(18)	(41)	(43)
HEPATOCELLULAR ADENOMA	2 (11%)	4 (10%)	8 (19%)
HEPATOCELLULAR CARCINOMA	1 (6%)		4 (9%)
URINARY SYSTEM			
#URINARY BLADDER	(15)	(32)	(35)
HEMANGIOMA		1 (3%)	
ENDOCRINE SYSTEM			
#PITUITARY	(5)	(22)	(14)
CHROMOPHOBE ADENOMA		1 (5%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(18)	(42)	(44)
ENDOMETRIAL STROMAL POLYP		1 (2%)	1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(18)	(42)	(45)
PAPILLARY ADENOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 22-2346	LOW DOSE 22-2344	HIGH DOSE 22-2342
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	3	4
MORIBUND SACRIFICE			3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	39	39
ANIMAL MISSING	2	8	4
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	13	23
TOTAL PRIMARY TUMORS	9	15	26
TOTAL ANIMALS WITH BENIGN TUMORS	4	7	11
TOTAL BENIGN TUMORS	5	8	12
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	7	14
TOTAL MALIGNANT TUMORS	4	7	14
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH NITHIAZIDE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH NITHIAZIDE

	CONTROL (UNTR) 11-1345	LOW DOSE 11-1343	HIGH DOSE 11-1341
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
NECROSIS, NOS			1 (2%)
#LUNG	(20)	(50)	(50)
CONGESTION, NOS	1 (5%)	1 (2%)	5 (10%)
PNEUMONIA, ASPIRATION			1 (2%)
BRONCHOPNEUMONIA, ACUTE			2 (4%)
PNEUMONIA, CHRONIC MURINE	2 (10%)	4 (8%)	3 (6%)
INFLAMMATION, GRANULOMATOUS			1 (2%)
HYPERPLASIA, ADENOMATOUS		2 (4%)	
#LUNG/ALVEOLI	(20)	(50)	(50)
EDEMA, NOS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(17)	(48)	(46)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
#SPLEEN	(20)	(50)	(49)
CONGESTION, CHRONIC	1 (5%)		
NECROSIS, NOS			1 (2%)
HEMOSIDEROSIS	1 (5%)		
LYMPHOID DEPLETION			1 (2%)
#SPLENIC CAPSULE	(20)	(50)	(49)
FIBROSIS, FOCAL		1 (2%)	

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE CI (CONTINUED)

	CONTROL (UNTR) 11-1345	LOW DOSE 11-1343	HIGH DOSE 11-1341
#MESENTERIC L. NODE	(20)	(48)	(49)
NECROSIS, NOS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(50)
THROMBUS, MURAL		1 (2%)	
PERIARTERITIS	1 (5%)		
#HEART/ATRIUM	(20)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	
THROMBUS, MURAL			2 (4%)
#AURICULAR APPENDAGE	(20)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	1 (2%)
#MYOCARDIUM	(20)	(50)	(50)
FIBROSIS	12 (60%)	22 (44%)	22 (44%)
#ENDOCARDIUM	(20)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
*PULMONARY ARTERY	(20)	(50)	(50)
MINERALIZATION		2 (4%)	2 (4%)
*HEPATIC VEIN	(20)	(50)	(50)
THROMBOSIS, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(49)
INFLAMMATION, CHRONIC			1 (2%)
HEPATITIS, TOXIC		1 (2%)	
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL		1 (2%)	
NECROSIS, ISCHEMIC	1 (5%)		
METAMORPHOSIS FATTY		3 (6%)	1 (2%)
BASOPHILIC CYTO CHANGE	1 (5%)	1 (2%)	1 (2%)
FOCAL CELLULAR CHANGE			2 (4%)
ATYPIA, NOS		1 (2%)	1 (2%)
ATROPHY, NOS			1 (2%)
LEUKEMOID REACTION		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1345	LOW DOSE 11-1343	HIGH DOSE 11-1341
HEMATOPOIESIS	1 (5%)		
#LIVER/CENTRILOBULAR	(20)	(50)	(49)
ATROPHY, NOS			1 (2%)
*BILE DUCT	(20)	(50)	(50)
FIBROSIS	1 (5%)	1 (2%)	5 (10%)
HYPERPLASIA, NOS	1 (5%)	5 (10%)	7 (14%)
#PANCREAS	(19)	(50)	(46)
PERIARTERITIS		1 (2%)	
#PANCREATIC ACINUS	(19)	(50)	(46)
ATROPHY, NOS		5 (10%)	2 (4%)
#STOMACH	(18)	(49)	(46)
AMYLOIDOSIS	3 (17%)	2 (4%)	3 (7%)
#COLON	(19)	(46)	(48)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
PARASITISM	4 (21%)	4 (9%)	3 (6%)
AMYLOIDOSIS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
HYDRONEPHROSIS		1 (2%)	
INFLAMMATION, CHRONIC	17 (85%)	35 (70%)	32 (64%)
GRANULOMA, NOS			2 (4%)
INFLAMMATION, FOCAL GRANULOMATOU	1 (5%)		
NEPHROPATHY, TOXIC	1 (5%)		3 (6%)
HEMOSIDEROSIS			2 (4%)
#URINARY BLADDER	(15)	(33)	(37)
DISTENTION			1 (3%)
HEMORRHAGE			1 (3%)
INFLAMMATION, ACUTE			1 (3%)
HYPERPLASIA, EPITHELIAL			1 (3%)
*URETHRA	(20)	(50)	(50)
ABSCESS, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(15)	(36)	(30)
HEMORRHAGIC CYST			1 (3%)

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL(UNTR) 11-1345	LOW DOSE 11-1343	HIGH DOSE 11-1341
ANGIECTASIS		1 (3%)	
#ADRENAL	(20)	(50)	(50)
MINERALIZATION		1 (2%)	
HEMORRHAGIC CYST			1 (2%)
ANGIECTASIS			1 (2%)
#ADRENAL CORTEX	(20)	(50)	(50)
CYTOPLASMIC VACUOLIZATION			2 (4%)
HYPERPLASIA, NODULAR			1 (2%)
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(20)	(50)	(50)
INFLAMMATION, ACUTE		1 (2%)	
#TESTIS	(20)	(50)	(50)
ATROPHY, NOS		1 (2%)	3 (6%)
#TESTIS/TUBULE	(20)	(50)	(50)
DEGENERATION, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	
*MESENTERY	(20)	(50)	(50)
INFLAMMATION, NECROTIZING		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONCLUDED)

	CONTROL(UNTR) 11-1345	LOW DOSE 11-1343	HIGH DOSE 11-1341
PERIARTERITIS			1 (2%)
NECROSIS, FAT		2 (4%)	3 (6%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMOID REACTION		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH NITHIAZIDE

	CONTROL (UNTR) 11-1346	LOW DOSE 11-1344	HIGH DOSE 11-1342
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
MINERALIZATION		1 (2%)	
ATELECTASIS	1 (5%)		1 (2%)
EMBOLUS, SEPTIC		1 (2%)	
CONGESTION, NOS	1 (5%)	1 (2%)	2 (4%)
EDEMA, NOS		2 (4%)	
EDEMA, INTERSTITIAL	1 (5%)		
HEMORRHAGE		1 (2%)	
BRONCHOPNEUMONIA, ACUTE			1 (2%)
PNEUMONIA, CHRONIC MURINE	2 (10%)	8 (16%)	10 (20%)
HYPERPLASIA, EPITHELIAL		1 (2%)	
HYPERPLASIA, ADENOMATOUS			2 (4%)
LEUKOCYTOSIS, NOS	1 (5%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(49)	(50)
CONGESTION, NOS		1 (2%)	1 (2%)
HEMOSIDEROSIS	3 (15%)	3 (6%)	1 (2%)
HYPERTROPHY, NOS			1 (2%)
HEMATOPOIESIS		1 (2%)	
#SPLENIC CAPSULE	(20)	(49)	(50)
FIBROSIS		1 (2%)	
#MANDIBULAR L. NODE	(19)	(48)	(48)
STEATITIS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1346	LOW DOSE 11-1344	HIGH DOSE 11-1342
#MESENTERIC L. NODE	(19)	(48)	(48)
HEMORRHAGE			1 (2%)
CIRCULATORY SYSTEM			
#AURICULAR APPENDAGE	(20)	(50)	(49)
THROMBOSIS, NOS		1 (2%)	
#MYOCARDIUM	(20)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	2 (4%)
FIBROSIS	9 (45%)	16 (32%)	18 (37%)
FIBROSIS, FOCAL	1 (5%)		
DEGENERATION, GRANULAR			1 (2%)
#ENDOCARDIUM	(20)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
CONGESTION, NOS			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
GRANULOMA, NOS			1 (2%)
DEGENERATION, HYDROPIC			2 (4%)
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL	1 (5%)		1 (2%)
NECROSIS, ISCHEMIC		1 (2%)	
METAMORPHOSIS FATTY	1 (5%)	5 (10%)	1 (2%)
BASOPHILIC CYTO CHANGE	1 (5%)	2 (4%)	7 (14%)
EOSINOPHILIC CYTO CHANGE			1 (2%)
ATYPIA, NOS	1 (5%)	1 (2%)	
LEUKOCYTOSIS, NOS	1 (5%)		
HEMATOPOIESIS			2 (4%)
#LIVER/CENTRILOBULAR	(20)	(50)	(50)
NECROSIS, NOS		1 (2%)	
ATROPHY, NOS			1 (2%)
*BILE DUCT	(20)	(50)	(50)
FIBROSIS		2 (4%)	
HYPERPLASIA, NOS	1 (5%)	2 (4%)	1 (2%)
#PANCREAS	(19)	(48)	(47)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1346	LOW DOSE 11-1344	HIGH DOSE 11-1342
#PANCREATIC ACINUS	(19)	(48)	(47)
METAMORPHOSIS FATTY			1 (2%)
ATROPHY, NOS	1 (5%)	1 (2%)	4 (9%)
ATROPHY, FOCAL	2 (11%)		
#STOMACH	(20)	(50)	(48)
AMYLOIDOSIS		2 (4%)	
HYPERPLASIA, FOCAL		1 (2%)	
#COLON	(20)	(49)	(49)
INFLAMMATION, NECROTIZING		1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
PARASITISM	3 (15%)	9 (18%)	14 (29%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
MINERALIZATION		1 (2%)	2 (4%)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, CHRONIC	6 (30%)	7 (14%)	6 (12%)
FIBROSIS	1 (5%)		
NEPHROPATHY, TOXIC		3 (6%)	1 (2%)
PIGMENTATION, NOS			1 (2%)
HEMOSIDEROSIS			1 (2%)
ATROPHY, NOS	1 (5%)		
HYPERPLASIA, TUBULAR CELL			1 (2%)
#KIDNEY/TUBULE	(20)	(50)	(50)
DILATATION, NOS			1 (2%)
CAST, NOS			1 (2%)
DEGENERATION, NOS	1 (5%)		
#KIDNEY/PELVIS	(20)	(50)	(50)
INFLAMMATION, ACUTE		1 (2%)	
#URINARY BLADDER	(14)	(37)	(37)
HYPERPLASIA, EPITHELIAL			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(39)	(47)
CYST, NOS	1 (6%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1346	LOW DOSE 11-1344	HIGH DOSE 11-1342
HEMORRHAGIC CYST		4 (10%)	4 (9%)
ANGIECTASIS			1 (2%)
#ADRENAL	(20)	(50)	(48)
CYTOLOGIC DEGENERATION			1 (2%)
#ADRENAL CORTEX	(20)	(50)	(48)
METAMORPHOSIS FATTY		1 (2%)	2 (4%)
PIGMENTATION, NOS			1 (2%)
CYTOLOGIC DEGENERATION		1 (2%)	1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
#THYROID	(19)	(38)	(26)
HYPERPLASIA, C-CELL		2 (5%)	2 (8%)
#THYROID FOLLICLE	(19)	(38)	(26)
DEGENERATION, CYSTIC			1 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CYSTIC DUCTS		1 (2%)	
HYPERPLASIA, CYSTIC		1 (2%)	
#UTERUS/ENDOMETRIUM	(19)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
HYPERPLASIA, CYSTIC	1 (5%)		
#OVARY	(19)	(50)	(48)
CYST, NOS		1 (2%)	1 (2%)
PAROVARIAN CYST	2 (11%)	1 (2%)	3 (6%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(48)
INFLAMMATION, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 11-1346	LOW DOSE 11-1344	HIGH DOSE 11-1342
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		3	2
AUTO/NECROPSY/HISTO PERF		2	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH NITHIAZIDE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH NITHIAZIDE

	CONTROL (UNTR) 22-2345	LOW DOSE 22-2343	HIGH DOSE 22-2341
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		3	6
ANIMALS NECROPSIED	20	47	44
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	47	44
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(20)	(44)	(44)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
#LUNG	(20)	(44)	(44)
THROMBOSIS, NOS			1 (2%)
CONGESTION, NOS			2 (5%)
HEMORRHAGE		2 (5%)	1 (2%)
BRONCHOPNEUMONIA, NOS		1 (2%)	
INFLAMMATION, INTERSTITIAL	1 (5%)	2 (5%)	2 (5%)
ABSCESS, NOS		1 (2%)	
PNEUMONIA, CHRONIC MURINE	2 (10%)	3 (7%)	7 (16%)
PERIVASCULAR CUFFING	1 (5%)	3 (7%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(46)	(43)
FIBROSIS			1 (2%)
NECROSIS, FOCAL			1 (2%)
HYPERPLASIA, NODULAR		1 (2%)	
HYPERPLASIA, LYMPHOID		2 (4%)	
HEMATOPOIESIS	1 (5%)	1 (2%)	
#LYMPH NODE	(19)	(43)	(43)
HYPERPLASIA, LYMPHOID		2 (5%)	
#MESENTERIC L. NODE	(19)	(43)	(43)
LYMPHANGIECTASIS	1 (5%)		

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2345	LOW DOSE 22-2343	HIGH DOSE 22-2341
INFLAMMATION, GRANULOMATOUS		1 (2%)	
HYPERPLASIA, RETICULUM CELL	1 (5%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(42)	(42)
EOSINOPHILIC CYTO CHANGE		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(46)	(43)
INFLAMMATION, NOS	1 (5%)		
ABSCESS, NOS		1 (2%)	
NECROSIS, NOS	1 (5%)		
NECROSIS, FOCAL	1 (5%)		
METAMORPHOSIS FATTY		1 (2%)	
BASOPHILIC CYTO CHANGE		1 (2%)	
FOCAL CELLULAR CHANGE		1 (2%)	
INCLUSION, CYTOPLASMIC			1 (2%)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	6 (14%)
HEMATOPOIESIS	1 (5%)		
#PANCREAS	(19)	(45)	(43)
HEMORRHAGE	1 (5%)		
HEMORRHAGIC CYST	1 (5%)		
DEGENERATION, NOS	1 (5%)		
#PANCREATIC ACINUS	(19)	(45)	(43)
ATROPHY, NOS			1 (2%)
#STOMACH	(19)	(45)	(44)
INFLAMMATION, FOCAL		1 (2%)	
#PEYERS PATCH	(19)	(44)	(43)
HYPERPLASIA, LYMPHOID		1 (2%)	3 (7%)
#COLON	(19)	(42)	(44)
PARASITISM	8 (42%)	14 (33%)	19 (43%)
URINARY SYSTEM			
#KIDNEY	(19)	(46)	(42)
MINERALIZATION	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 22-2345	LOW DOSE 22-2343	HIGH DOSE 22-2341
HYDRONEPHROSIS		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	2 (11%)		
#KIDNEY/CAPSULE	(19)	(46)	(42)
INFLAMMATION, ACUTE		1 (2%)	
#URINARY BLADDER	(16)	(36)	(40)
CYST, NOS			1 (3%)
INFLAMMATION, CHRONIC	1 (6%)		
ENDOCRINE SYSTEM			
#ADRENAL	(17)	(42)	(41)
INFLAMMATION, ACUTE		1 (2%)	
#ADRENAL CORTEX	(17)	(42)	(41)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
#THYROID	(14)	(29)	(31)
CYSTIC FOLLICLES			1 (3%)
ATROPHY, FOCAL			1 (3%)
HYPERPLASIA, FOLLICULAR-CELL		1 (3%)	
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(20)	(47)	(44)
INFLAMMATION, ACUTE		1 (2%)	
LIPOGRANULOMA			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(46)	(44)
MINERALIZATION	7 (35%)	11 (24%)	4 (9%)
PSAMMOMA BODIES	2 (10%)	8 (17%)	20 (45%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 22-2345	LOW DOSE 22-2343	HIGH DOSE 22-2341
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(47)	(44)
INFLAMMATION, NOS	1 (5%)		
NECROSIS, NOS	1 (5%)		
*MESENTERY	(20)	(47)	(44)
NECROSIS, FAT	1 (5%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		5	2
ANIMAL MISSING/NO NECROPSY		2	6

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH NITHIAZIDE

	CONTROL (UNTR) 22-2346	LOW DOSE 22-2344	HIGH DOSE 22-2342
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	2	8	4
ANIMALS NECROPSIED	18	42	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	18	42	45
INTEGUMENTARY SYSTEM			
*SKIN	(18)	(42)	(45)
ABSCESS, CHRONIC		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(16)	(42)	(43)
INFLAMMATION, ACUTE			1 (2%)
#LUNG	(16)	(42)	(43)
THROMBUS, ORGANIZED			1 (2%)
CONGESTION, NOS			1 (2%)
INFLAMMATION, INTERSTITIAL		5 (12%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	1 (6%)	4 (10%)	13 (30%)
PERIVASCULAR CUFFING		3 (7%)	1 (2%)
FOAM-CELL			1 (2%)
MEGAKARYOCYTOSIS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(17)	(39)	(43)
MEGAKARYOCYTOSIS		1 (3%)	
HYPERPLASIA, LYMPHOID		7 (18%)	2 (5%)
#LYMPH NODE	(18)	(38)	(40)
HYPERPLASIA, LYMPHOID		3 (8%)	
#MESENTERIC L. NODE	(16)	(38)	(40)
HYPERPLASIA, LYMPHOID			1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(18)	(36)	(40)
FIBROSIS		1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2346	LOW DOSE 22-2344	HIGH DOSE 22-2342
*CORONARY ARTERY	(18)	(42)	(45)
SCLEROSIS		1 (2%)	
*PULMONARY ARTERY	(18)	(42)	(45)
HYPERTROPHY, NOS		2 (5%)	
HYPERPLASIA, NOS		2 (5%)	
DIGESTIVE SYSTEM			
#LIVER	(18)	(41)	(43)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
PERIVASCULAR CUFFING		1 (2%)	
HYPERPLASIA, FOCAL			2 (5%)
HEMATOPOIESIS	1 (6%)	1 (2%)	
#SMALL INTESTINE	(18)	(40)	(44)
ABSCESS, NOS			1 (2%)
#COLON	(18)	(38)	(43)
INFLAMMATION, NECROTIZING			1 (2%)
PARASITISM	2 (11%)	8 (21%)	12 (28%)
URINARY SYSTEM			
#KIDNEY	(18)	(41)	(42)
MINERALIZATION			3 (7%)
HYDRONEPHROSIS	1 (6%)	1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (6%)		
INFLAMMATION, CHRONIC		1 (2%)	
PERIVASCULAR CUFFING		1 (2%)	
#URINARY BLADDER	(15)	(32)	(35)
HYPERPLASIA, LYMPHOID			1 (3%)
ENDOCRINE SYSTEM			
#ADRENAL	(18)	(37)	(36)
HEMORRHAGE		1 (3%)	
METAMORPHOSIS FATTY		1 (3%)	
LIPOIDOSIS		2 (5%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2346	LOW DOSE 22-2344	HIGH DOSE 22-2342
REPRODUCTIVE SYSTEM			
#UTERUS	(18)	(42)	(44)
HYDROMETRA			1 (2%)
CYST, NOS	1 (6%)		3 (7%)
HEMORRHAGE		1 (2%)	
#CERVIX UTERI	(18)	(42)	(44)
HYPERTROPHY, NOS		1 (2%)	
#UTERUS/ENDOMETRIUM	(18)	(42)	(44)
INFLAMMATION, NOS	1 (6%)	5 (12%)	1 (2%)
HYPERPLASIA, NOS		7 (17%)	5 (11%)
HYPERPLASIA, CYSTIC	9 (50%)	23 (55%)	13 (30%)
#OVARY	(16)	(36)	(42)
CYST, NOS		1 (3%)	3 (7%)
FOLLICULAR CYST, NOS	1 (6%)	6 (17%)	3 (7%)
PAROVARIAN CYST	4 (25%)	4 (11%)	4 (10%)
HYPERPLASIA, CYSTIC			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(16)	(41)	(45)
MINERALIZATION	3 (19%)	10 (24%)	1 (2%)
PERIVASCULAR CUFFING		1 (2%)	
PSAMMOMA BODIES	2 (13%)	5 (12%)	8 (18%)
SPECIAL SENSE ORGANS			
*EYE	(18)	(42)	(45)
CATARACT			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL WALL	(18)	(42)	(45)
INFLAMMATION, CHRONIC		1 (2%)	

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2346	LOW DOSE 22-2344	HIGH DOSE 22-2342
*PERITONEAL CAVITY	(18)	(42)	(45)
PERIARTERITIS		1 (2%)	
*MESENTERY	(18)	(42)	(45)
NECROSIS, FOCAL	1 (6%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(18)	(42)	(45)
AMYLOIDOSIS		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	2	2
ANIMAL MISSING/NO NECROPSY	2	8	4
AUTOLYSIS/NO NECROPSY			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

Review of the Bioassay of Nithiazide* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

October 25, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of Nithiazide for carcinogenicity.

The reviewer for the report on the bioassay of Nithiazide said that the results indicated the compound to be carcinogenic in treated male mice but that the evidence for its carcinogenicity in females was "dubious." Although a statistically significant increase in mammary tumors in treated female rats was observed, he questioned its biological meaningfulness because of the variability in incidence of the tumor type. The reviewer concluded that Nithiazide was not carcinogenic in either sex of treated rats. After briefly describing the experimental design, he noted a nine week interruption in treatment due to the lack of Nithiazide. Despite this shortcoming and the small number of control animals, he said that the study still appeared to be adequate. Based on the results of the bioassay, the reviewer said that Nithiazide should be considered to pose, at most, a slight carcinogenic risk to humans. There was no objection to a recommendation that the report on the bioassay of Nithiazide be accepted as written.

Clearinghouse Members Present

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Kenneth Wilcox, Michigan State Health Department

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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